PROPERTIES OF CELL WALL-ASSOCIATED ENZYMES IN THE BOUND AND FREE STATE

INTRODUCTION

Determination of enzymatic properties of cell wall-associated enzymes are usually performed on salt-solubilized or free enzymes in solution (2). Since limited studies have been reported on cell wall-bound enzymes *in situ* (1, 2, 3), we have investigated the *in situ* properties of two cell wall-associated enzymes. In one case (β-N-acetylglucosaminidase), the properties of a bound versus free enzyme were directly compared. In the second case (acid phosphatase), the effects of cations on the *in situ* enzyme activity was determined.

MATERIALS AND METHODS

Cell walls were isolated from Russet potato tubers and highly purified as described previously (4). Purified cell walls were either extracted with 3 M LiCl for 15 hr at 4°C or assayed without treatment. Salt extracted enzymes were concentrated in an Amicon ultrafiltration apparatus (PM 10 membrane) and dialyzed for 2 days with water containing 1 mM 2-mercaptoethanol. β–N-acetyl-glucosaminidase activity (β-GlcNAcase) and acid phosphatase activity (APase) were determined as described (5) except the APase assay was performed in 50 mM Na-acetate buffer at pH 4.5. When cell wall enzymes were assayed *in situ*, 0.1 to 0.5 mg dry weight cell wall were used per assay at 38°C and enzymatic reactions were stopped with 1 M Na_2CO_3. The assay tubes were centrifuged to pellet the cell walls and samples were read at 405 nm.

RESULTS AND DISCUSSION

Properties of bound versus free β-GlcNAcase. Approximately 30% of the total cellular β-GlcNAcase activity was associated with the cell wall. The cell wall-bound activity was almost completely solubilized (over 95%) with 3 M LiCl and therefore provided an ideal enzyme to study *in situ* (bound) versus the solubilized (free) condition. The bound enzyme had maximum activity at pH 5.0 while the free enzyme had maximum activity at pH 4.7. Most striking was the fact that the bound enzyme had little or no activity at pH 3.5 while the free enzyme at this pH had one half the maximum

activity observed. When kinetic studies were performed at pH 5.0, the bound enzyme had a Km value of 570 μM and the free enzyme had a Km value of 280 μM. These results indicated there are wall effects which influence enzyme activity.

The effects of cations on cell wall-associated acid phosphatase. Cell walls with low Ca^{2+} content were isolated from sycamore cell suspensions and shown to contain an APase with pronounced negative cooperative kinetics (1). In the presence of 1 mM $CaCl_2$, the negative cooperativity was drastically reduced and approached Michaelis-Menten kinetics. By increasing the ionic strength of the bulk phase, simple Michaelis-Menten kinetics was observed (1). Negative cooperativity was thought to be a result of the electrostatic repulsion of the negatively charged substrate from the polyanionic cell wall. Increased ionic strength suppressed the repulsion. Their theory on the ionic control of cell wall-associated enzymes relies on the existence of an electrostatic potential difference between the inside and outside of the cell wall which is regulated by Ca^{2+} (1). This theory has been recently extended to explain the control of cell wall expansion (5).

We observed negative cooperativity with potato tuber cell wall-associated APase; however, 1 mM $CaCl_2$ had no effect on this kinetic property. On the other hand, 1 mM $MgCl_2$ eliminated the negative cooperativity and generated Michaelis-Menten kinetics. This result indicated that the theory of enzymatic regulation by the electrostatic potential may not be sufficient to account for the specificity of Mg^{2+} over Ca^{2+} on the cell wall-APase of potato tubers. Further studies will be necessary to extend or modify the ideas presented by Ricard et al. (1, 5).

LITERATURE CITED

1. CRASNIER M, A-M MOUSTACAS, J RICARD 1985 Electrostatic effects and calcium ion concentration as modulators of acid phosphatase bound to plant cell walls. Eur J Biochem 151: 187-190
2. GOLDBERG R 1985 Cell-wall isolation, general growth aspects. *In* Modern Methods of Plant Analysis Vol. 1. Cell components. HF Linskens, JF Jackson, eds., Springer-Verlag, New York, pp 1-30
3. NAGAHASHI G, PM BARNETT, S-I TU, J BROUILLETTE 1986 Purification of primary cell walls from corn roots: Inhibition of cell wall-associated enzymes with indolizidine alkaloids. *In* Regulation of carbon and nitrogen reduction and utilization in maize. JC Shannon, DP Knievel, CB Boyer, eds, Waverly Press, Baltimore, pp 289-293
4. NAGAHASHI G, TS SEIBLES 1986 Purification of plant cell walls: Isoelectric focusing of $CaCl_2$ extracted enzymes. Protoplasma 134: 102-110
5. RICARD J, G NOAT 1986 Electrostatic effects and the dynamics of enzyme reactions at the surface of plant cells. I. A theory of the ionic control of a complex multi-enzyme system. Eur J Biochem 155: 183-190